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DIETARY INCLUSION OF *MONDIA WHITEI* ROOT POWDER FOR MALE *OREOCHROMIS SHIRANUS*

Nakawoombe M[1,2*], Kapute F[3], Nababi J[7], Alunga GL[1,8], Zaabwe T[1,6], Luwaga D[9,10], Lazaro WJ[5] and K Mzengereza[4]



Milliam Nakawoombe

*Corresponding author email: millysekitto@gmail.com & milliam.nakawoombe@agriculture.go.ug
ORCiD: https://orcid.org/0009-0008-7753-7996

[1]Department of Fisheries and Aquatic Sciences, Mzuzu University, Private Bag 201, Luwinga, Mzuzu 2, Malawi

[2]Regulation and Quality Assurance Department, Directorate of Fisheries Resources, Ministry of Agriculture, Animal Industry and Fisheries, Plot 16-18, Lugard Avenue, P.O. Box 102, Entebbe, Uganda

[3]Directorate of Research, Mzuzu University, Private Bag 201, Luwinga, Mzuzu 2, Malawi

[4]Faculty of Environmental Sciences, Mzuzu University, Private Bag 201, Luwinga, Mzuzu 2, Malawi

[5]Aquaculture and Fisheries Science Department, Lilongwe University of Agriculture and Natural Resources, Bunda College Campus, P.O. Box 219, Lilongwe, Malawi

[6]National Agricultural Research Organization, Bulindi Zonal Agricultural Research and Development Institute, Box 101, Hoima, Uganda

[7]Department of Chemistry, Mzuzu University, Luwinga, Mzuzu 2, Malawi

[8]Centre de Recherches Universitaires du Kivu (CERUKI) ISP, Bukavu, Sud-Kivu, Democratic Republic of the Congo

[9]Department of the Built Environment, Mzuzu University, Private Bag 201, Luwinga, Mzuzu 2, Malawi

[10]Department of Environmental Studies, Faculty of Agriculture, Uganda Martyrs University, Kampala, Uganda

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ABSTRACT

Most extensive and semi-intensive fish farmers in Malawi are adopting formulating feed on-farm to reduce feed costs. To improve aquafeed quality, research on environmentally friendly and cost-effective feed additives has increased. Therefore, this study assessed the effect of incorporating *Mondia whitei* root powder as an aromatic feed additive on the proximate composition and palatability of feed in male *Oreochromis shiranus* to improve feed effectiveness. The morphological characteristics and market acceptability of experimental fish were also assessed. A completely randomized design was used with five dietary treatments of *M. whitei* inclusion (0, 10, 20, 30, and 40%) for male *O. shiranus* (experimental mean initial body weight: 80 g). Proximate composition (AOAC methods) was used to determine the nutrient profile of *M. whitei* root powder and experimental diets. Feed palatability was assessed through video-based observation of fish feeding behavior and quantification of uneaten feed following air-drying. Fish morphological characteristics and market acceptability were assessed through consumer feedback. One-way analysis of variance (ANOVA) at the 5% significance level was used for data analysis. The polynomial trend analysis assessed the potential dose-dependent responses of *Mondia whitei* inclusion levels. Proximate composition had significant differences for crude protein and fat among diets ($p \leq 0.001$), and a significant difference ($p \leq 0.05$) in feed palatability was also observed across all treatments. The 20% inclusion diet showed the highest palatability, indicated by a shorter latency time (20 min). Fish growth was comparable across treatments, with no significant differences in percentage weight gain and specific growth rate (SGR, %/day) ($p > 0.05$). In addition, there was no significant linear or quadratic relationship between the inclusion level of *Mondia whitei* and SGR ($p > 0.05$) across treatments, indicating that *Mondia whitei* inclusion did not negatively influence fish growth performance. The condition factor (K) values were comparable across treatments, suggesting normal physiological conditions and growth. No significant changes in morphological appearance, texture, and color appeal were observed ($p > 0.05$), with the consumer feedback rating all groups as acceptable and marketable. Therefore, *Mondia whitei* root powder inclusion in male *Oreochromis shiranus* diets improved the nutritional profile and feed palatability. It did so without impairing the fish's morphological characteristics or growth performance, showing its potential as a reliable phytogenic additive in formulated fish feed.

Key words: *Mondia whitei*, herbs, feed palatability, *Oreochromis shiranus,* Aquaculture nutrition, tilapia

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INTRODUCTION

Tilapia in the genus *Oreochromis* is among the most farmed freshwater fish species worldwide [1]. The widespread culture is attributed to their typical features: high palatability, rapid growth, relatively good growth performance in captivity, and the ability to withstand various environmental stressors [2]. In Malawi, *Oreochromi*s species are the main cultured fish species. *Oreochromis shiranus (O. shiranus)* is one of the four tilapia species (the others are: *O. karongae, O. mossambicus, and Coptodon rendalli*) widely raised in ponds. Locally known as "makumba", *O. shiranus* is a delicacy that is highly preferred by consumers and has substantial market demand within Malawi [3]. However, the production of *O. shiranus*, like that of other cultured tilapia species, is often constrained by high production costs associated with expensive aquaculture inputs, particularly feed. Feed plays an important role in *Oreochromis* aquaculture productivity, with feed and feeding expenses accounting for ≥60% of total production costs [4]. Although protein sources remain the major cost driver in aquafeed formulations, tilapia, being an omnivorous species with predominantly herbivorous feeding habits, can efficiently utilize plant-based protein sources. This makes tilapia an ideal species for evaluating alternative feed ingredients, such as *Mondia whitei* root powder, as potential dietary supplements. In addition, species-specific nutritional requirements, particularly for broodstock that need higher protein and lipid levels to support reproductive performance, complicate formulating [4]. Although alternative plant protein sources such as cottonseed meal, canola meal, and groundnut cake are used in aquafeed formulations, their effectiveness in fish broodstock diets remains limited due to poor digestibility, antinutritional factors, and imbalanced amino acid profiles [5].

Beyond nutrient balance is palatability, an important determinant of feed success. Fish must readily accept feeds to ensure nutrient intake. Feed aroma (in water) is one of the major characteristics that affects palatability; the aroma and taste of a diet have important roles in palatability and fish feeding motivation, especially during the introduction of new feed formulations [6]. Aromatic plant inclusions can improve diet acceptance and reduce feed wastage, provided their effects on proximate composition and feed acceptability are understood. Technologies aimed at enhancing feed aroma are being explored to improve feed utilization, with phytogenic additives often working better [7].

Phytogenic additives are defined as natural feed additives derived from plants or plant-based materials, including herbs, spices, essential oils, extracts, and powders incorporated into animal diets to improve feed quality, palatability, digestion, health, and overall performance [8]. As extensive and semi-intensive fish farmers increasingly adopt formulating feed on-farm to reduce feed-related costs, most of these formulated feeds tend to show sinking characteristics due to limited

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technology. Therefore, improving their palatability provides an added advantage because improved palatability promotes rapid feed acceptance before pellets sink, minimizing feed wastage [9]. Extensive research has, therefore, been done on phytogenic additives because of their ability to enhance nutritional quality, improve palatability, and boost overall feed utilization efficiency [8]. Studies on the inclusion or supplementation with ginger (*Zingiber officinale*) and garlic (*Allium sativum*) showed significant improvements in growth, immune response, and feeding behavior of cultured fish [10,11].

Mondia whitei (Hook. f.) Skeels, belonging to the family Apocynaceae, is widely distributed in sub-Saharan Africa and is generally regarded as safe for traditional use [12]. Different parts of the plant are utilized as food, medicine, and herbal preparations; however, its commercial cultivation remains limited [12]. Due to the extensive indigenous knowledge, particularly that associated with the root bark, *M. whitei which is* widely utilized in areas where it exists as a medicine, a tonic, an appetite stimulant, and an aphrodisiac. It is known by various local names across Africa. In western Kenya, it is referred to as “ogombo” or “mukombero”, “mulondo” in Uganda, “limte”, “nkang bongo”, “yang”, or “la racine” in Cameroon, “gondolosi” in Malawi, and “mbombongazi” in Tanzania [13]. *M. whitei* roots are locally accessible through herbal markets and traditional plant collectors, although availability may fluctuate seasonally due to increasing exploitation and habitat disturbance. The price ranges approximately between 1 and 2 US dollars/kg of the roots. *M. whitei* is characterized by aromatic roots that are rich in bioactive compounds such as alkaloids, saponins, flavonoids, and phenolic compounds [12]. It has been studied in humans, rodents, poultry, and livestock [12]. Its characteristic aroma and potential bioactivity support *M. whitei* as a potential phytogenic feed additive to complement conventional feed ingredients.

M. whitei is characterized by aromatic roots that are rich in bioactive compounds such as alkaloids, saponins, flavonoids, and phenolic compounds [12]. It has been investigated in humans, rodents, poultry, and livestock [12]. Its characteristic aroma and diverse bioactive constituents support its potential as a phytogenic feed additive to complement conventional feed ingredients rather than replace them.

However, despite documented reports of its proximate composition, as shown in Table 6 [14,15], limited information was found on the use of *M. whitei* root in aquaculture feeds and its phytogenic potential in fish. This study, therefore, evaluated the effects of *M. whitei* inclusion in fish feed as an aromatic herb on the proximate composition and palatability of experimental diets for the improvement of male *Oreochromis shiranus* broodstock nutrition and feed palatability. This work constitutes the feed quality and palatability component of a broader investigation into the aphrodisiac potential of *M. whitei* in male *O. shiranus* broodstock.

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MATERIALS AND METHODS

The experiment used a single-factor design, with dietary inclusion levels of *M. whitei* root powder (0, 10, 20, 30, and 40%) as the independent variable. The dependent variables included the proximate composition and palatability of the experimental diets, as well as the growth performance parameters, morphological characteristics, and acceptability of the experimental fish.

Preparation of diets

Fresh *M. whitei* roots, 10 kg (average length 25–35 cm, diameter 2–3 cm, mean fresh weight 180 ± 20 g/root) were collected from the Mzuzu region where the plant is naturally abundant. The local guides helped with the root recognition and harvesting. The harvested roots were carefully packed and transported to the Mzuzu Chemistry Laboratory for further processing. At the laboratory, the root bark was manually peeled off the root stem using stainless-steel knives to obtain the outer bark (indigenous knowledge is about the plant's root bark). The root bark samples were then sliced into smaller pieces (2–3 cm) using these knives and cleaned with distilled water. The cleaned samples were air-dried indoors in a well-ventilated room away from direct sunlight at 25 ± 2 °C for 14 days to ensure proper drying while minimizing photodegradation [12]. The dried material was ground into a fine powder using a stainless-steel grinder (Model 800Y; capacity 800 g; 220 V; 1400 W; 30 000 rpm; Yongkang Bochu Electric Appliance Co., Ltd., China) and sieved through a 0.5 mm mesh stainless-steel sieve to ensure uniform particle size. ~3 kg of dried *M. whitei* root powder was obtained. The *M. whitei* root powder was stored in airtight plastic containers at room temperature for 1 wk in the laboratory. Commercial fish feed pellets (Grower's Feed, 32 % crude protein floating pellets (5 mm) from Novatek Animal Feeds, Lusaka, Zambia)) were similarly ground, sieved, and stored together with the powder. Thereafter, the experimental diets were formulated at inclusion levels of 0 (control), 10, 20, 30, and 40% as shown in Table 1. The mixtures were blended and extruded using a FAO FATT PANMO laboratory pelletizer Jiangxi Hongxing machinery limited liability company, Dongxiang, Jiangxi, China) (3–5 HP, belt-driven, capacity ~100 kg/h). Pelleting was done at ambient temperature (22 ± 2 °C) under the mechanical pressure of the extrusion chamber, processed for ~20 min/batch. The resulting pellets were air-dried indoors to a constant weight for 5 days, packaged in one-kg black polyethylene bags tied manually without air removal. The pellets were stored at room temperature for 1 wk. The diets constituted the 5 dietary treatments used during the feed palatability assessment. Recognizing that reprocessing can alter nutrient quality, proximate composition analysis was done to determine the actual nutrient profile of the experimental diets. In addition, after the experimental period, the fish condition factor was calculated (as detailed under the subsection on fish morphological appearance

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and consumer feedback) to assess the overall well-being of the fish, ensuring that both feed quality and biological responses were accounted for.

Feed Proximate Analysis

Proximate composition analysis (in triplicate) on experimental diets (diet 1-5) that represented the dietary treatments 0, 10, 20, 30 and 40%, and *M. whitei* powder was done in the Mzuzu Chemistry laboratory. The AOAC guidelines [16], as applied by Ssewagudde [17], were followed. Moisture content was determined using AOAC Method 934.01 by oven-drying samples at 105°C for 24 hr. Ash content was determined according to AOAC Method 942.05 by incineration in a muffle furnace at 650°C for ~6 hr. Crude fat was determined using Soxhlet extraction (AOAC Method 920.39) with n-hexane as the extraction solvent. Crude protein was determined using the Kjeldahl method (AOAC Method 954.01). The samples for crude protein determination were digested using a mixed catalyst comprised of potassium sulphate, copper sulphate, and selenium metal in a ratio of 8:1:1 (w/w/w). Nitrogen content was converted to crude protein using a factor of 6.25. Carbohydrate content was estimated by difference.

The fish feeding trial setup

A 5-wk feeding trial was done at the Mzuzu Aquaculture Center using indoor glass tanks (60 × 50 × 30 cm) with a working volume of 60 L of static potable tap water, settled for 24 h before use. Treatments were assigned following a completely randomized design. Randomization was done manually by sequentially allocating fish from a common holding tank to treatment tanks, ensuring equal probability of assignment. The five dietary treatments were tested. Experimental fish were obtained from the Mzuzu Aquaculture Center, from a batch of ~120 fry at 4–5 months of age (40–60 g). Male and female fish were identified and separated with the help of the experienced farm technician based primarily on genital papilla morphology, supported by external secondary sexual characteristics where necessary. The fish were subsequently reared separately on a commercial diet until they attained an average body weight of approximately 80 g (6–7 months old). The same feeding frequency and ration were maintained throughout the feeding trial, with the commercial diet replaced by the respective experimental diets. Twenty (20) male *Oreochromis shiranus* with an average initial body weight of 80 g were stocked at two fish/tank and separated by transparent dividers to minimize aggression. The transparent dividers were fabricated from locally available plastic egg trays, which were cut and modified to fit the length and width of the tanks. Twenty male *O. shiranus* were randomly distributed among ten tanks, with two fish per tank. Each dietary treatment was replicated in two tanks, resulting in four fish per treatment (n = 4). Each treatment was replicated twice, requiring a total of 10 tanks. Fish initial body weight (80 g) was used to determine the quantities of feed at 3% of this initial

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body weight and was kept constant throughout the trial. This approach minimized handling stress and was appropriate given that the study focused on feed palatability rather than growth performance. Water quality data collection was done daily at 10:00 am using a portable multi-parameter water quality meter (ProQuatro, YSI, Yellow Springs, OH, USA). The following water quality parameters were measured: pH, water temperature (°C), total dissolved solids (TDS, mg/L), salinity (ppm), conductivity (µS/cm), and dissolved oxygen (DO, mg/L). The meter was calibrated according to the manufacturer's instructions before measurements were taken each sampling day.

Feed palatability assessment

During the first week of the feeding trial, feed palatability was assessed over a 7-day period using video-based observations. The fish were starved for 24 hr to standardize hunger levels across treatment groups. Three underwater action cameras were mounted externally on the tanks to capture feeding behavior (one Dragon Touch Vision 3 Pro, Dragon Touch Inc., Shenzhen, Guangdong, China, and two AKASO Brave 4 cameras, AKASO Technology LLC, Shenzhen, China). The cameras were positioned to record each fish separately. Although palatability observations were done for 1 hr after feed introduction, each camera recorded for ~2 h to allow time for camera setup, fish acclimation following disturbance, feeding observation, and removal of uneaten feed using a 0.5 mm mesh net. Due to the limited number of cameras available, recordings were done sequentially. To maintain similar observation conditions across treatments, at least one fish from each treatment was recorded on the same day. This procedure was repeated for 7 consecutive days until all four fish from each treatment had been assessed. 1 hr after the feed was given, the uneaten feed and the cameras were collected from each tank. The uneaten feed was recovered from each tank partition using a 0.5-mm mesh sieve. Although the pellets softened during immersion, they generally remained identifiable and recoverable. Minor feed losses due to leaching and handling during recovery may have occurred and represent a potential source of measurement error. The recovered feed was air-dried at ambient laboratory temperature (~21–22°C) during a cold season (May–June) and subsequently weighed. The cameras automatically saved the recordings as consecutive 5-min video clips, and although the total recording period was approximately 2 h, only the 12 clips corresponding to the 60-min period after feed introduction were selected for feeding behavior assessment and palatability analysis. The recordings were first reviewed using VideoLAN Client (VLC) Media Player, where they were converted from MPEG-4 Part 14 (MP4) to Audio Video Interleave (AVI) format to ensure compatibility with ImageJ (National Institutes of Health, Bethesda, MD, USA). The AVI files were subsequently examined frame by frame in ImageJ to facilitate detailed observation of feeding behavior. Feed acceptance was estimated as the proportion

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of feed consumed relative to the amount initially offered. The following behavioral parameters were obtained: latency time (s) (time from feed introduction to first bite), feeding intensity (number of feeding attempts/min), and % feed acceptance, i.e., proportion of feed consumed within 60 min:

$$\%\text{Feed acceptance} = \frac{\text{Total feed administered} - \text{Uneaten feed}}{\text{Total feed administered}} \times 100 \quad [18]$$

These feeding responses were used as indicators of diet palatability, with shorter latency, higher feeding intensity, and greater feed acceptance reflecting stronger feeding responses. The diets were categorized as highly palatable (+++), moderately palatable (++), or poorly palatable (+) based on observed feeding responses and feed consumption. This scoring approach was guided by Kumar *et al.* [19], who described palatability in terms of feed acceptance and animals' feeding responses to a diet.

Fish morphological appearance and consumer feedback

After the 1-wk palatability assessment, experimental feeding continued for an additional 4 wk under the same feeding conditions. At the end of the feeding period, all fish were individually removed, measured for total length (cm) using a 50 cm ruler and body weight (g) using a precision scale ROHS kitchen scale (capacity: 5000 g; readability: 0.1 g; maximum capacity: 5000 g; Shenzhen Kakakele Technology Limited, Shenzhen, Guangdong, China). Fish were handled individually and kept out of water only for the time required to obtain the measurements (~2 min), after which they were immediately returned to their respective tanks. The length and weight data were used to calculate Fulton's condition factor (K) as an indicator of fish well-being.

$$K = \frac{W}{L^3} \times 100$$

where: K = condition factor, W = body weight of the fish (in g), L = total length of the fish (in cm) and 100 = a scaling factor.

After the collection of the quantitative data, fish samples from the different treatments were qualitatively analyzed. The fish were divided into two batches. Two fish from each treatment were used to determine the morphological appearance, and the other two for consumer feedback.

For morphological appearance, the 10 samples were further divided into two groups. The first group had 5 fish randomly collected from the holding treatment tanks used, temporarily sedated by immersion in cold water containing clove extract for 15 mins. In the laboratory of the Mzuzu Aquaculture Centre, the 5 anesthetized fish were placed on white laboratory trays for a visual examination of the color appeal, body color intensity, fin appearance, scale integrity, apparent condition, and general

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appearance. Each attribute was scored on a 5-point ordinal scale (1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = excellent). Scoring was done independently by a panel of 10 farmers, using a standardized checklist (Appendix 1). The panelist farmers were customers of the Mzuzu Aquaculture Centre who had previously purchased fish or fingerlings from the centre. The trays were rotated among panelists until each participant had independently assessed all fish. A second batch of 5 fish, obtained from the same treatment tanks as the first group, was temporarily sedated using the same procedure and placed individually on separate coded white laboratory trays. Unlike the first batch, which was used for morphological appearance assessment, this group was used exclusively for market perception assessment to avoid repeated evaluation of the same fish. Market perception was assessed using a structured questionnaire (Appendix 2), which evaluated perceived marketability, willingness to purchase, perceived quality, and expected market price. The assessment was conducted by an independent panel of 10 fishmongers (5 males and 5 females) who were regular customers purchasing table fish from the Mzuzu Aquaculture Centre during fish market days. Each panelist independently completed one questionnaire for every fish assessed. Following completion of the assessments, the fish were humanely euthanized and disposed of in accordance with the standard operating procedures of the Mzuzu Aquaculture Centre.

Consumer feedback evaluation was by an independent panel of 10 fish farmers randomly selected from the customer records of the Mzuzu Aquaculture Centre. These participants were different from those involved in the morphological appearance assessment. The remaining 10 fish were collected from the treatment tanks, humanely euthanized, and each boiled in a coded saucepan over a firewood stove for 20 min. The cooked fish were allowed to cool for approximately 5 min before being transferred to a correspondingly coded serving tray to maintain sample identity. Each panelist was then randomly assigned one whole fish for evaluation. Color appeal, aroma, taste, texture, and overall acceptability were assessed using a structured evaluation checklist (Appendix 3). Each attribute was rated on a 5-point hedonic scale ranging from 1 (not acceptable) to 5 (highly acceptable).

Statistical analysis

All statistical analyses and data visualization were done using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA), Microsoft Excel 2021 (Microsoft Office LTSC Professional Plus, Microsoft Corp., Redmond, WA, USA), and R version 4.4.3 (R Core Team, Vienna, Austria). Video tracking and morphometric image analyses were done using Fiji (ImageJ distribution, version 1.54p, National Institutes of Health, Bethesda, MD, USA) [20]. Data were analyzed using one-way analysis of variance (ANOVA) after testing for normality and homogeneity of variances. Where significant differences occurred, treatment means

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were separated using Tukey's HSD test at $p \leq 0.05$. Since dietary inclusion levels represented an increasing gradient, polynomial trend analysis was done for specific growth rate (SGR) to assess possible linear and quadratic responses to increasing *M. whitei* inclusion.

$$SGR = \frac{\ln(W_t) - \ln(W_0)}{t} \times 100$$

where (W_t) = final weight, (W_0) = initial weight, and (t) = duration in days. This analysis assessed possible linear and quadratic responses to increasing *M. whitei* inclusion levels.

RESULTS AND DISCUSSION

Influence of dietary *M. whitei* on nutrient composition of diets

The experimental diets (Diets 1–5) differed in proximate composition from the commercial feed and *M. whitei* root powder (Table 2).

Protein content differed significantly among experimental diets ($p < 0.001$), with all *M. whitei* inclusion diets (10–40%) showing significantly higher protein levels than both commercial feed and *M. whitei* powder (Tukey's HSD, $p \leq 0.05$). Crude protein was highest in the 10% (47.7%) and 20% (45 %) inclusion diets, followed by a decline at the 30% and 40% inclusion levels. The commercial feed (control diet) and *M. whitei* powder had crude protein contents of 28.6% and 10.9%, respectively. Although this trend differed from the theoretical expectation based on the crude protein contents of the individual ingredients, it should be noted that crude protein was determined by the Kjeldahl method on the finished pelleted diets rather than estimated from ingredient composition. Consequently, the reported values represent the analyzed composition of the processed feed matrix following mixing, pelleting, and drying, which is the standard approach for evaluating formulated feeds [16,21]. Despite the unexpectedly high crude protein values at the moderate inclusion levels, the subsequent decline at higher inclusion levels suggests that increasing replacement of the commercial feed with *M. whitei* did not result in a proportional increase in the crude protein content of the finished diets. Therefore, under the formulation conditions of the present study, moderate inclusion levels (10–20%) produced the most favorable nutritional profile and provided a useful basis for interpreting the subsequent palatability responses.

Fat content varied significantly across treatments. The commercial feed (control diet) had the lowest value (3.5%), while the 40% inclusion diet showed the highest (6.4%). *M. whitei* root powder contained 6.0% fat, and diets with higher inclusion levels, particularly 30 and 40%, showed correspondingly elevated fat content. The slightly higher crude fat values observed at the 30 and 40% inclusion levels are unlikely to

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represent true lipid enrichment attributable to *M. whitei* alone. Rather, they likely reflect normal analytical variation associated with proximate analysis of processed feed matrices [16]. Statistical analysis confirmed significant differences among treatments ($p \leq 0.001$). Tukey's HSD test indicated that *M. whitei* powder, as well as the 30 and 40% inclusion diets, had significantly higher fat content than the control diets (adjusted $p \leq 0.05$). The roots of *M. whitei* are known to contain an essential oil at ~1–1.2% and a fixed (nonvolatile) oil content of ~2.8% [22]. Thus, the increase in fat content at higher *M. whitei* experimental diets indicated the plant's contribution to the fat/lipid profile [13].

While fat and protein content increased, the ash content had a contrasting trend. Ash content differed significantly among experimental diets ($p \leq 0.001$). The control diet (0%) had the highest ash content (6.9%), while *M. whitei* powder had the lowest value (5.0%). The 10–40% groups did not differ significantly (all adjusted $p > 0.05$). This indicates that the control diet maintained adequate mineral levels for fish health, compared to *M. whitei* powder and the experimental diets. But the experimental diets' ash content range fell within an acceptable range, because a 5-7% ash level is recommended for balanced tilapia diets [23].

The present study reported a high moisture content of *M. whitei* powder (13.2%), which may reflect incomplete drying and the ability of dried plant powders to absorb atmospheric moisture during storage, a characteristic reported for many plant-derived feed ingredients [24,25]. On the other hand, the lower moisture content in the control diet (8.4%) indicates a pelletized feed with a good shelf life and a reduced risk of microbial spoilage. El-Sayed [26] recommended that the moisture content of fish feed remain <10%, which all diets met.

The reduction of carbohydrate content observed in lower inclusion diets (32.5 %) for 10% and (36.0 %) for 20%, with elevated protein content (47.7 %) for 10% and (45 %) for 20%, is not necessarily disadvantageous, because tilapia generally utilize dietary protein efficiently for growth and other physiological functions [27]. The observed nutrient shift towards higher crude protein at moderate inclusion levels suggests that dietary inclusion of *M. whitei* improved the aroma and nutritional composition of the formulated diets, a characteristic that may be beneficial in broodstock nutrition where adequate dietary protein is required to support gonadal growth and reproductive development [27,28].

Effect of dietary *M. whitei* root powder inclusion on feed palatability in *O. shiranus*

The experimental diets showed comparable feed palatability across treatments, indicating that the inclusion of *M. whitei* root powder did not impair feed palatability. However, some variation in the amount of feed eaten was observed among treatments ($p = 0.0005$, Tukey's HSD, $p \leq 0.05$) (Figure 1). On a 1.2 g ration, mean

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uneaten feed was lowest for the 20% inclusion (0.41 g) and highest for the 10 and 30% inclusions (0.62 g). Fish fed the 20% *M. whitei* diet also showed a shorter latency time (1200 s). Importantly, in 10–40% inclusion treatments, direct ingestion, defined as immediate biting or swallowing upon feed contact, was not observed, but fish were observed to consistently approach the feed. The fish swam towards the feed and spent several s in the feeding zone. Frequent nibbling and active downward swimming toward the feed were also noted. The control treatment (0% inclusion) showed an alternate feeding behavior as the fish were observed to swim near the surface and collect the pellets since they had floating pellets. From the data collected, the highest feeding intensity (5.0 ± 0.3 attempts/hr) and palatability score (+++) occurred with the 20% inclusion, followed by moderate responses (++) across the remaining treatments (Table 3). Despite the behavioral differences between sinking and floating feed, all *M. whitei* inclusion diets were consumed at levels comparable to the control diet. This is of particular interest since most on-farm-made feed is sinking; thus, inclusion of *M. whitei* can be incorporated into such diets without compromising feed acceptance by the fish[9]. The comparable feed acceptance observed between the *M. whitei* inclusion diets and the control suggests that inclusion of *M. whitei* maintained the palatability of the formulated diets. The plant's aromatic compounds, as documented in the literature [13], together with the lipid content of the diets, may have contributed to this response. Additionally, the volatile components in *M. whitei* may have enhanced feed odor and taste, contributing to behavioral responses such as reduced latency and increased feeding intensity, thereby indicating feed attractiveness [2].

The water quality parameters remained within the recommended ranges for *O. shiranus* culture throughout the trial. The study was conducted during the cool season in Mzuzu, Malawi, when ambient temperatures are typically below 20°C. Accordingly, the mean water temperature was 19.0 ± 0.1°C, which is slightly below the reported optimum range of 22–28°C for *O. shiranus* [3]. The remaining water quality parameters, including pH (7.5 ± 0.1), TDS (91 ± 7 mg/L), salinity (61 ± 5 ppm), conductivity (140 ± 13 µS/cm), and dissolved oxygen (7.3 ± 0.04 mg/L), remained stable throughout the study. Maintaining these parameters within acceptable limits is important because water quality influences fish feeding behavior, metabolism, and energy allocation [26].

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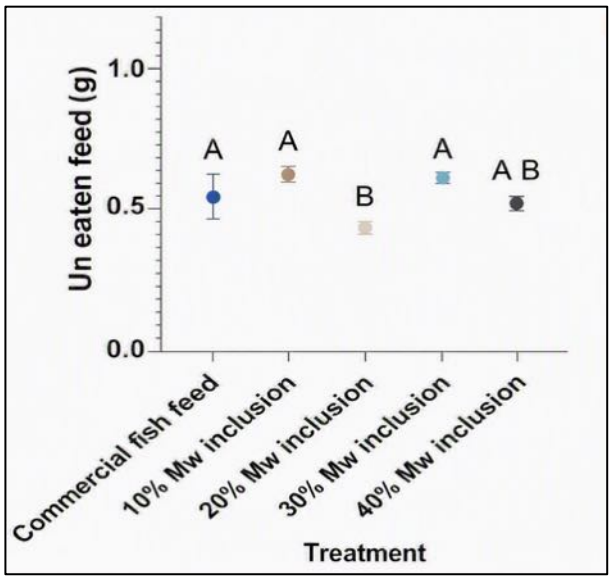


Figure 1: The feed palatability test plotted as uneaten feed against the different treatments

[1]Mean uneaten feed (g) was used as an indicator of feed acceptance among fish offered diets containing different levels of *M. whitei*. Error bars represent standard error. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$).

Fish growth performance

Growth performance indicators, including percentage weight gain and SGR, remained comparable across treatments (Table 4). One-way ANOVA showed no significant differences among treatments for either ($p \geq 0.05$). Polynomial regression of SGR against dietary inclusion level yielded a slope of 0.00217 ($p = 0.545$, $R^2 = 0.029$), indicating no significant linear relationship among the varying inclusion levels of *M. whitei* diets. The comparable growth performance observed across treatments suggests that dietary *M. whitei* inclusion did not impair nutrient utilization or normal physiological development in *O. shiranus*. These results are in line with Van Hai [29], who reported that phytogenic additives, including garlic (*Allium sativum*), neem (*Azadirachta indica*), and turmeric (*Curcuma longa*), enhanced immune responses and antibody production in fish without compromising growth performance.

Fish morphological appearance and consumer feedback

Fish fed *M. whitei* diets maintained firm texture, symmetrical fins, and robust body profiles. Color appeal and scale integrity scores were comparable across treatments, with 20 and 30% *M. whitei* dietary inclusion treatments showing enhanced pigmentation for visual appeal (“golden and fresh,” “nice shape”). The 10 % group showed uniform pigmentation, glossy scales, and a healthy sheen (fish’s natural coloration and body integrity). In comparison, the 40% group retained marketability

[1]

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despite mild fading (lighter pigmentation and reduced surface sheen). Sensory panelists rated all treatments as marketable and acceptable, with no negative remarks on taste nor texture (Figure 2a and 2b). One-way ANOVA showed no significant differences in condition factor (K) (Table 5) across treatments ($p > 0.05$), indicating that *M. whitei* inclusion did not adversely affect somatic growth or body robustness, competitiveness, and marketable features of *O. shiranus*. The visual appeal and enhanced pigmentation at 20 and 30% *M. whitei* inclusion diets may be attributed to the increased protein and fat content at these levels, since proteins are known to support tissue development and scale integrity, while dietary lipids contribute to skin luster and pigment deposition [30–32]. Hence, these results are consistent with previous reports indicating that *M. whitei* is safe for consumption [12,13].

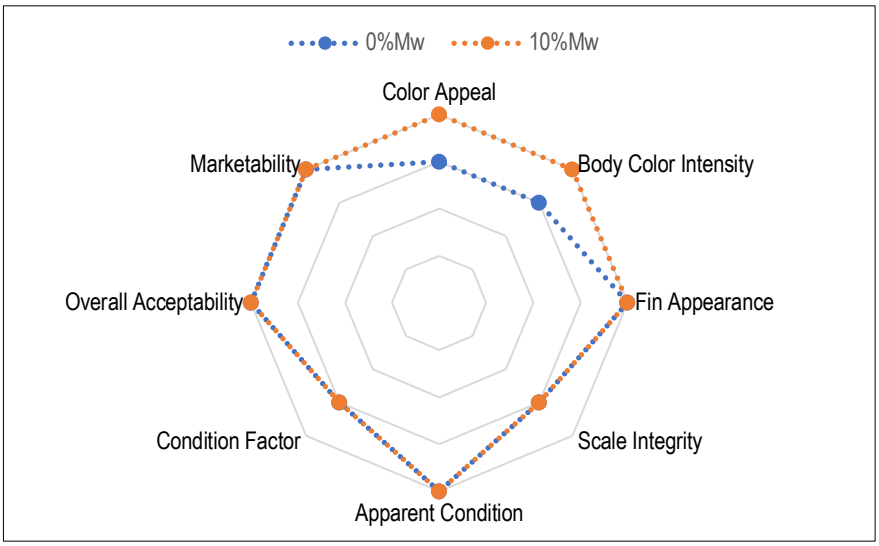


Figure 2a: Morphological appearance and consumer feedback scores of male *O. shiranus* fed on 0 and 10% *M. whitei* powder inclusion diets

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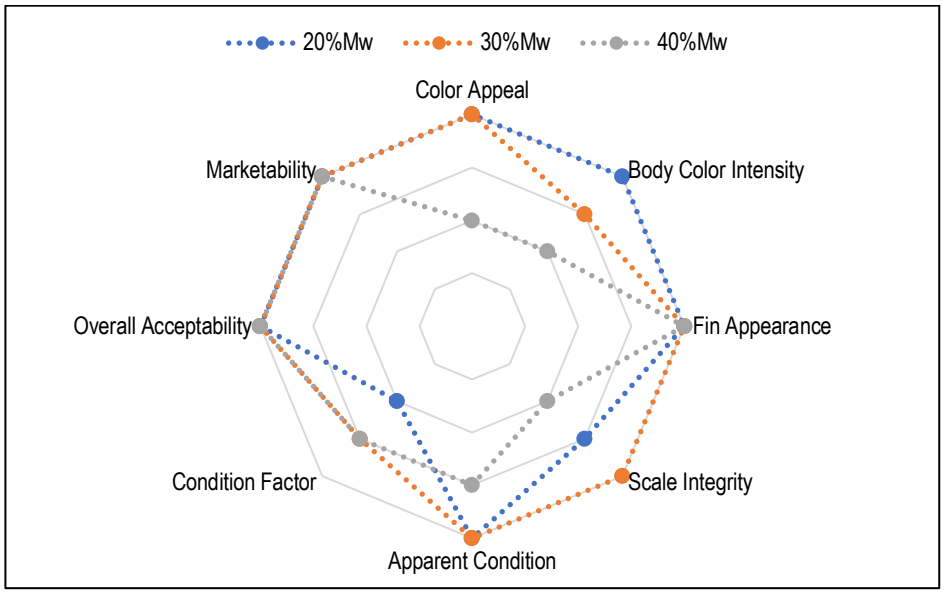


Figure 2b: Morphological appearance and consumer feedback scores of male *O. shiranus* fed on 20, 30, and 40% *M. whitei* powder inclusion diets

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The inclusion of *M. whitei* root powder in the diets of *O. shiranus* altered the proximate composition of the formulated diets, particularly by increasing the analyzed crude protein and crude fat contents. Growth performance, body robustness, condition factor, external morphology, and marketable characteristics of the fish were not adversely affected. Among the inclusion levels evaluated, the 20% diet showed the highest feed palatability, reflected by greater feed acceptance and lower uneaten feed. The ability of *M. whitei* root powder to be finely ground and readily incorporated into formulated diets further highlights its potential as a practical phytogenic feed additive.

The results of this study suggest that a 20% dietary inclusion of *M. whitei* root powder represents a promising inclusion level for further evaluation in *O. shiranus* diets. However, before its adoption by fish farmers, further studies should evaluate its performance over longer feeding periods, using larger fish populations under farm conditions. Additional research should also optimize the formulation of *M. whitei*-based diets and assess their physical stability, storage stability, and economic feasibility for commercial aquaculture.

Study limitations

The present study provides baseline information on the proximate composition of formulated diets, feed palatability, and morphological responses of male *O. shiranus* fed diets containing *M. whitei* root powder. However, several limitations should be acknowledged. The experiment was conducted during a relatively cool period (19–20°C), which may have influenced fish metabolism and feeding behavior. Growth

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performance and condition factor were evaluated using wet weight measurements only, as dry weight was not determined, limiting direct comparison of body composition and nutrient retention. In addition, the relatively short feeding period and limited sample size may have reduced the ability to detect subtle long-term physiological responses. Finally, the characterization of *M. whitei* was limited to proximate composition, and the bioactive compounds responsible for the observed responses were not quantified.

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Table 1: Ingredient composition of experimental diets

Ingredients	Prepared diets				
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)	Diet 5 (40%)
Mondia whitei root powder (g)	0	100	200	300	400
Cassava flour (g)	0	30	30	30	30
Commercial feed (g)	1000	870	770	670	570

Table 2: Proximate composition (mean ± SE) of each of the experimental diets and *M. whitei* root powder

Proximate Composition (%)	Experimental diets					*Mondia whitei* root powder
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)	Diet 5 (40%)	
Ash	6.9±0.01	6.0±0.1	6.3±0.2	6.3±0.4	6.3±0.1	5.0±0.02
Crude fat	3.5±0.3	4.4±0.0	4.3±0.5	6.1±0.4	6.4±0.2	6.0±0.2
Crude protein	28.6 ±0.4	47.7±0.1	45±1	37.3±0.1	31±1	10.9 ±0.1
Moisture content	8.4±0.1	9.4±0.0	8.7±0.3	8.6±0.1	8.3±0.1	13.2 ±0.1
Carbohydrates*	52.5 ±0.4	32.5±0.3	36±2	42±1	47.7±0.5	9 ±0.3

*= by difference

Table 3: Feeding behavior indices of male *O. shiranus* fed on experimental diets

Treatment group	Latency time (s)	Feeding intensity (attempts/hr±SE)	Feed acceptance (%)	Palatability score
0% Mw inclusion	1210	4.0±0.2	58±4	++
10% Mw inclusion	1800	3.5±0.1	50±3.1	++
20% Mw inclusion	1200	5.0±0.3	67±3	+++
30% Mw inclusion	1440	3.5±0.1	50±2	++
40% Mw inclusion	1320	4.5±0.1	58±2	++

Mw =*Mondia whitei*

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Table 4: Growth performance of *O. shiranus* fed diets with varying *M. whitei* inclusion levels

Treatment (% *M. whitei* inclusion)	% Weight Gain (mean ± SE)	SGR (mean ± SE)
0	40±6	0.70 ± 0.12
10	41±9	0.73 ± 0.20
20	50±3	0.66 ± 0.09
30	48±2	0.65 ± 0.06
40	40±5	0.85 ± 0.02

Table 5: Condition factor (K) values with mean ± SD of the male *O. shiranus*

Treatment group	Condition Factor (K)
0% Mw	1.6±0.3
10% Mw	1.5±0.0
20% Mw	1.4±0.2
30% Mw	1.5±0.0
40% Mw	1.8 ± 0.1

fed on experimental diets

Table 6: Comparative Proximate Composition of *M. whitei*: Fruit [15] and Root [14]

Parameter	Fruit (%) FW Chinonyerem & Maryjane [15]	Root (g/100 g) DW Abdou Bouba *et al.* [14]
Moisture	88.2	10.5
Ash	0.6	10.5
Crude fat	1.2	2–3
Crude fiber	1.4	12–14
Crude protein	2.5	4.7
Carbohydrates	5.0	~5
Energy value	40.8 Kcal/100 g	~300 Kcal/100 g

FW = fresh weight; DW = dry weight

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APPENDICES

Appendix 1: Fish morphological appearance checklist

Respondent Information

Respondent No.: _____, Age: _____, Gender: _____, Sample (Tray) Code: _____

Instructions: Please evaluate the fish presented and tick (✓) the score that best reflects your opinion.

Scale:1 = Very Poor 2 = Poor 3 = Fair 4 = Good 5 = Excellent

Attribute	0	10	20	30	40
Color Appeal	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5
Body Color Intensity	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5
Fin Appearance	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5
Scale Integrity	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5
Apparent Condition (visible health and vitality)	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5
General appearance (attractiveness)	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5	☐1 ☐2 ☐3 ☐4 ☐5

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Appendix 2: Fish market perception questionnaire

Respondent Information

Respondent No.: _____, Age: _____, Gender: _____, Sample (Tray) Code: _____

Instructions: Please evaluate the fish presented and tick (✓) the score that best reflects your opinion.

Scale:1 = Very Poor 2 = Poor 3 = Fair 4 = Good 5 = Excellent

Market Attribute	1	2	3	4	5
Scale condition and integrity	☐	☐	☐	☐	☐
Overall appearance	☐	☐	☐	☐	☐
Willingness to purchase	☐	☐	☐	☐	☐
Overall market acceptability	☐	☐	☐	☐	☐

Price Expectation

What price would you be willing to pay for this fish?

(Malawian Kwacha) MWK: _____

Additional Comments

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Appendix 3: Boiled fish consumer evaluation checklist

Respondent No.: _____, Sample Code: _____, Date: _____

Instructions: Please provide and rate each attribute of the boiled fish sample by ticking (✓) the score that best reflects your opinion.

Scale:1 = Not acceptable, 2 = Slightly acceptable, 3 = Moderately acceptable, 4 = Acceptable

5 = Highly acceptable

Sensory Attribute	**1**	**2**	**3**	**4**	**5**
Color appeal of the boiled fish	☐	☐	☐	☐	☐
Aroma of the boiled fish	☐	☐	☐	☐	☐
Taste of the boiled fish	☐	☐	☐	☐	☐
Texture of the boiled fish	☐	☐	☐	☐	☐
Overall acceptability	☐	☐	☐	☐	☐

Additional Comments

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